Biospectra : Vol. 8(2), September, 2013, Spl. issue. pp 173-180
An International Biannual Refereed Journal of Life Sciences

Plant Sciences
Abst.No.MBC- 09

ISSN : 0973-7057

Brassinosteroids treatment to *Brassica juncea* L. plants under Copper metal stress affects sugar and lipid metabolism.

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Received 30th July, 2013; Revised 25th August, 2013

Abstract : Brassinosteroids (BRs) are a class of polyhydroxysteroids and have been reported to counteract both abiotic and biotic stresses in plants. We studied the ameliorative effect of 24-Epibrassinolide (24-EpiBr) on *Brassica juncea*L. under copper (Cu) metal stress. Growth parameters, sugars and lipids classes were analysed. In most of the cases these parameterswere increased by 24-EpiBr in Cu stressed plants and the stress was ameliorated. So, 24-EpiBr treatment can be used to increase the plant survival and yield in Cu rich soils. 24-EpiBr has good future prospects as it can play a significant role in enhancing plant production and protection in Cu contaminated soil.

Key Words: Copper metal stress, 24-Epibrassinolide, Sugars, Lipids

INTRODUCTION

Heavy metal contamination is one of the major problems due to industrial processes, agricultural practices and disposal of industrial and agricultural wastes etc[1]. Cu is one of the heavy metal, which is an essential metal also required for normal plant growth and development[2]. But it has detrimental effects at higher concentrations and its day by day increasing concentration in the environment is posing threat to all the life forms including plants and animals. The main sources which contaminate the soil with Cu include pig and poultry manures, metal finishing, by-products of microelectronics and pesticides[3].

Excess of Cu is toxic to plants. Its inhibitory effect on growth had been noted even after one day of treatment[4]. A long exposure to Cu decreased chlorophyll concentration, which was associated with the destruction of inner structure of chloroplasts[5]. The excess of Cu also affected enzymes activities[6]. Sugars played role in osmoregulation and an increase in sugars was observed under heavy metal treatment[7]. Various lipid classes showed decrease on heavy metal treatments (Cu and Cd) in *Lycopersicon esculentum*[8]. BRs are a class of polyhydroxysteroids and are widely distributed in the plant kingdom. They are involved in a number of plant processes such as cell division, cell elongation, growth and reproductive development, vascular differentiation[9,10,11]. It shows their importance in plant metabolism. They protect plants from various stresses such as heavy metal stress[12,13], heat stress[14], salt stress[15]. Keeping in view these stress ameliorative properties of BRs, the present piece of work was undertaken to study the its effects on growth parameters, sugars and lipids metabolism in *B.juncea*L., an edible crop, under Cu stress.

MATERIALS AND METHODS

Seeds of *B. juncea* L. were procured from Punjab Agriculture University, Punjab, India. Seeds were surface sterilized using 0.4 % sodium hypochlorite, followed by

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rinsing with distilled water. The seeds were then given pre-soaking treatment in different 24-EpiBr concentrations (10^{-11}, 10^{-9} and 10^{-7} M). These seeds were sown in different blocks of field, prepared using random block design, containing different concentrations of Cu (0, 0.25, 0.5 and 0.75 mM). Plants were harvested on 60 days after sowing.

Growth Analysis

Shoot lengths and root lengths of harvested plants were analysed.

Biochemical Analysis

Extraction: *B.juncea* L. leaves were extracted with ethanol in boiling water bath. Extracts were dried to aqueous syrup and volume was made to 100 ml with distilled water.

Estimation of various sugars

Total Sugars

Total sugars were estimated using method of Dubois[16]. To the test extract, 5 % phenol and 5 ml conc. H_2SO_4were added. Absorbance was taken at 490 nm against blank. The concentration of total sugars was determined from the standard curve prepared using glucose standards.

Reducing Sugars

These were determined using the method of Nelson[17]. Five reagents were prepared.Reagent A: Dissolved anhydrous sodium carbonate, potassium sodium tartarate, sodium bicarbonate and anhydrous sodium sulphate in 100 ml distilled water. Reagent B: $CuSO_45H_2O$ was dissolved in 4 ml distilled water followed by the addition of conc. H_2SO_4. Reagent C: It was prepared by mixing reagent A and B in the ratio 25:1. Reagent D: Ammonium molybdate was dissolved in 50 ml distilled water and 2.3 ml of conc. H_2SO_4 was added. To this sodium arsenate solution in distilled water was added. Procedure: Test extracts were taken in test tubes, to them 1 ml of reagent C was added. The tubes were heated in boiling water bath. 1 ml of reagent D was added. The volume was made to 10 ml. Absorbance was measured at 520 nm against blank. The concentration of reducing sugars was determined from standard curve of glucose standards.

Glucose

Glucose was determined by the method of Gascon and Lampen[18].Three types of reagents were prepared. Reagent A: For it to 0.1 M potassium phosphate buffer (pH 7.0) 50 mg of glucose oxidase was added followed by addition of 2.5 mg of peroxidase. Reagent B: 30 mg of o-dianisidine was added to methanol. The solution was stored in an amber coloured bottle. Reagent C: It was prepared by the mixing of 6 ml of reagent A, 3 ml of reagent B and 51 ml of 45 % of glycerol. Procedure: Took test extract in a test tube. To it 1 ml of solution C was added. After it 2 ml of 2N HCl was added. Absorbance was taken at 540 nm. The amount of glucose was determined from the standard curve of glucose.

Free Fructose

The difference in the amounts of total reducing sugars and the glucose was taken as the value for fructose.

Lipids

Extraction: Method used by Kates[19] was used.*B.juncea* L. leaveswere blended with methanol-chloroform (2:1, v/v).Homogentae was filtered. Filter residue was washed with 30 ml of methanol-chloroform (2:1, v/v). Filterates were combined and were taken in a separatory funnel. To the mixture was added 50 ml of chloroform and 58 ml of water.Chloroform layer was withdrawn and was dried. The residual lipids were dissolved in chloroform.

Estimation of various lipids

Phospholipids

Method of Ames and Dubin[20]was used. Procedure: Test extract was taken in a test tube and chloroform was evaporated. To each test tube, 10 % magnesium nitrate was added. The contents were heated on low flame and then on strong flame.0.5 N HCl was added to each test tube. The tubes were heated in boiling water bath. This process converted the organic phosphorous into inorganic form. Then to estimate the phosphorous, the tubes were cooled. For it a reagent 'A' was prepared by mixing ascorbic acid (10% w/v) and ammonium molybdate(0.42 w/v in 1N H_2SO_4). To the sample solution obtained above 4.2 ml of reagent 'A' was added and incubated at 45°C. Absorbance was taken at 820 nm against a blank. The concentration of phosphorous in the samples was measured from a standard curve of potassium dihydrogen orthophosphate.

Glycolipids

These were determined with the method of Dubois[16]. Lipidsample was taken in a test tube. The chloroform was

evaporated. 2 ml of distilled water was added and shaken. 1 ml of 5 % phenol was 5 ml of conc. H_2SO_4 were added. The absorbance was taken at 490 nm against a blank. The concentration of glucose was measured from standard curve of glucose.

Total Sterols

Method of Sperry and Webb[21] was used. Lipid sample was taken in a test tube. To it 5 ml of chloroform, 1 ml of acetic anhydride and 0.1 ml of conc. H_2SO_4 were added. Absorbance was taken at 625 nm against blank. Concentration of total sterols was measured from a standard curve of ergosterol.

Esterified Sterols

Lipid sample was taken a test tube and1 % of digitonin solution was added to it. The mixture was evaporated. After it 3 ml of petroleum ether was added. The tubes were heated in water bath such that half of the solvent got evaporated. The esterified sterols were then measured using the the same method used for the estimation of total sterols.

Statistical Analysis

All experiments were carried out in triplicates. Data was presented as mean ± standard error. The data was subjected to two-way analysis of variance (ANOVA). The data was considered significant at *p* d" 0.05.

RESULTS AND DISCUSSION

The growth parameters – shoot length and root length decreased significantly as compared to control with the Cu treatment. However supplementation of Cu with 24-EpiBr reduced the suppressing of shoot length and root length by Cu. Shoot length was observed to be lowest (20.5 ± 0.67 cm) at 0.75 mM Cu as compared to control (30.537 ± 0.817 cm) and showed significant improvement by the application of 10^{-7} (33.163 ± 0.381 cm), 10^{-9} (31.212 ± 0.646 cm) and 10^{-11} (29.65 ± 0.531 cm) 24-EpiBr alone. Greatest amelioration was found in the case when 0.5 mM Cu treatment was supplemented with 10^{-7}M 24-EpiBr, the enhancement in the shoot length was 28.412 ± 0.33 cm as compared to control (**Table 1**).

Similar trend was observed in the case of root length which was observed to be lowest (7.063 ± 0.044 cm) at 0.75 mM Cu treatment as compared to control (9.563 ± 0.016 cm). There was enhancement in the root length when Cu treatment was supplemented with 24-EpiBr. Maximum stress amelioration was observed when 10^{-7} M 24-EpiBr treatment was given along with 0.5 mM Cu treatment (**Table 2**).

The effect of Cu and 24-EpiBr treatment was studied on sugars. Total sugars increased with the Cu treatment. They increased further when Cu treatment was supplemented with 24-EpiBr treatment. Maximum increase (2.987 ± 0.103mg) in the total sugars was observed when 0.50 mM Cu was supplemented with 10^{-7} M 24-EpiBr (**Table 3**).

Reducing sugars also showed an increasing trend with Cu treatment as compared to control. When alone Cu treatment was given, maximum increase (2.644 ± 0.024 mg) was observed in 0.75 mM Cu treatment as compared to control (1.905±0.01mg). Reducing sugars content showed further increase when Cu treatment was supplemented with 24-EpiBr, with maximum increase (2.902±0.095 mg) observed when 0.5 mM Cu was supplemented with 10^{-7} M 24-EpiBr (**Table 4**).

However, glucose levels declined with Cu treatment as compared to control (1.057 ± 0.005 mg), minimum decrease (0.782 ± 0.014 mg) observed at 0.75 mM Cu treatment. When 24-EpiBr treatments were given alone, there was some increase in glucose content as compared to control. The reducing effect of Cu was overcome significantly when Cu was supplemented with 24-EpiBr, with maximum increase (1.125 ± 0.016mg) observed when 0.5 mM Cu was supplemented with 10^{-7} M 24-EpiBr (**Table 5**).

Fructose content showed significant increase with the Cu treatment as compared to control (0.848 ± 0.007 mg), with the maximum increase (1.862 ± 0.01 mg) observed in 0.75 mM Cu treatment. When 24-EpiBr treatment was given alone, fructose levels were close to the control value. When Cu was supplemented with 24-EpiBr, there was an increase in the fructose content as compared to control and Cu-treatment alone. Maximum fructose content (1.921 ± 0.005 mg) was observed when 0.75 mM Cu was supplemented with 10^{-9} M 24-EpiBr (**Table 6**).

Phospholipids showed significant decrease with the Cu treatment as compared to control (0.102 ± 0.001 mg), with maximum decrease (0.074 ± 0.002 mg) observed in 0.75 mM Cu treatment. However when Cu treatment was

supplemented with 24-EpiBr there was significant increase in the phospholipids content, with maximum increase (0.131 ± 0.002 mg) observed when 0.50 mM Cu treatment was supplemented with 10^{-7} M 24-EpiBr (**Table 7**).

However, glycolipids content showed a significant increase with Cu treatment as compared to control (0.402 ± 0.004 mg), with maximum increase (0.528 ± 0.002 mg) observed in 0.75 mM Cu treatment. Supplementing the Cu treatment with 24-EpiBr, further increased the glycolipids content, with maximum increase (0.582 ± 0.006 mg) observed in 0.5 mM Cu treatment supplemented with 10^{-7} M 24-EpiBr (**Table 8**).

Conversaly, a significant decrease was noted in the total sterols content with the Cu treatment as compared to control. When 24-EpiBr treatments were given alone, total sterols contents were close to the control value (4.804 ± 0.043 mg). When Cu treatment was supplemented with 24-EpiBr treatment, an increase was observed as compared to the Cu treatment given alone, with maximum increase (5.68 ± 0.02 mg) observed in 0.5 mM Cu treatment supplemented with 10^{-7} M 24-EpiBr (**Table 9**).

Esterified sterols content showed a significant decrease with Cu treatment as compared to control (2.522 ± 0.022 mg), with maximum decrease (2.022 ± 0.016mg) observed in the case of 0.75 mM Cu. However, when Cu treatment was supplemented with 24-EpiBr, an increase was observed in the content of esterified sterols, with maximum level (2.293 ± 0.027 mg) to be noted in 0.25 mM Cu treatment supplemented with 10^{-7} M 24-EpiBr (**Table 10**).

In the present study, 24-EpiBr has been observed to ameliorate Cu stress in *B.juncea* L. The stress ameliorative effect of 24-EpiBr under heavy metal stress have also been studied by Sharma and Bhardwaj[22], Choudhary[23] and Arora[24]. 24-EpiBr improved plant growth by increasing shoot length and root length. It might be due to decreased Cu metal uptake[22].

Total soluble sugars increased with increasing levels of Cu treatment in two maize cultivars[7]. Total reducing sugars also showed increase with the increase in concentration of Cu metal treatment in two rice cultivars[7] and in *Phaseolusvulgaris* exposed to Co, Ni and Zn stress[25]. The increase might be due to the role of sugars in osmoregulation[26]. Soluble sugars allowed plants to maximize enough storage reserves which supported basal metabolism under stress conditions[27]. However, glucose levels decreased on Pb treatment as compared to control in *Phaseolusmungo*[28] and in *Vignaradiata* due to Cu stress[29]. But, the second reducing sugar -fructose showed an increase in rice roots treated with Cr[30]. In the present study, glucose decreased while fructose increased on Cu treatment. The decrease in glucose/fructose ratio under stress conditions reflected an altered carbon flux[31].Total sugars and total reducing sugars showed an increase on treatment with 28-homobrassinolide in *Pelargonium graveolens* (L.) Herit[32].Total reducing sugars showed an increase on treatment with 28-homobrassinolide and 24-EpiBr in *Raphanus sativus*[33]. The increase might be due to the enhancement of photosynthetic capacity of plants on 28-homobrassinolide and 24-Epibrassinolide application[33].

Phospholipids, important membrane lipids, decreased under heavy metal (Cu and Cd) stress in *L. esculentum*[8]; under Cd stress in *Pisum sativum*[34]; under salt stress in *Spartina patens*[35]. Normal translocation of electrons was affected under heavy metal stress, which resulted in free radical production that led to lipid peroxidation[36]. However, glycolipids showed an increase on Cu treatment[35,37]. As suggested by Williams[38], glycolipids played a role in stabilizing the interaction of bulk lipids and proteins by providing a more efficient sealing of proteins into the lipid head groups. If this case was considered, the increase in glycolipids in *B.juncea*L. under Cu stress stabilized the structural integrity of membranes, when they were exposed to excess Cu. As observed for phospholipids, total sterols also showed decrease under Cu treatment in marsh plant *S. patens*[35].

24-EpiBr treatment to plants reduced MDA content in *Oryzasativa* L. under salt stress[15], in *R.sativus* under Ni treatment[39]. This reduction in lipid peroxidation might be responsible for the increase in all the lipid classes by the treatment of 24-EpiBr under Cu stress.

Table 1 Effect of 24-EpiBr on shoot length (cm) in 60 days old plants of *B. juncea* L. plants grown under Cu stress (mean± S.E.).

Treatments	Control	0.25 mM Cu	0.5 mM Cu	0.75 mM Cu
Control	30.537 ± 0.817	23.425 ± 1.027	22.075 ± 0.397	20.5 ± 0.67
10^{-11}	29.65 ± 0.531	27.45 ± 0.203	25.85 ± 0.04	24.238 ± 0.67
10^{-9}	31.212 ± 0.646	28.212 ± 0.17	27.05 ± 0.196	25.3 ± 0.099
10^{-7}	33.163 ± 0.381	28.288 ± 0.487	28.412 ± 0.33	25.413 ± 0.07

Treatment (Cu): F-ratio$_{(3 X 47)}$ = 147.8624 (Significant at p d" 0.05);Dose (24-EpiBr): F-ratio$_{(3 X 47)}$ = 64.9015 (Significant at p d" 0.05);Cu X 24-EpiBr: F-ratio$_{(9 X 47)}$ = 4.8519 (Significant at p d" 0.05).

Table 2 Effect of 24-EpiBr on root length (cm) in 60 days old plants of *B. juncea* L. plants grown under Cu stress (mean± S.E.).

Treatments	Control	0.25 mM Cu	0.5 mM Cu	0.75 mM Cu
Control	9.563 ± 0.016	8.013 ± 0.018	7.15 ± 0.126	7.063 ± 0.044
10^{-11}	9.975 ± 0.098	9.1 ± 0.117	8.463 ± 0.099	8.413 ± 0.241
10^{-9}	10.5 ± 0.42	9.238 ± 0.047	8.525 ± 0.038	8.463 ± 0.076
10^{-7}	10.988 ± 0.025	10.038 ± 0.08	9.15 ± 0.056	8.625 ± 0.018

Treatment (Cu): F-ratio$_{(3 X 47)}$= 193.63 (Significant at p d" 0.05); Dose (24-EpiBr): F-ratio$_{(3 X 47)}$= 113.1717 (Significant at p d" 0.05);Cu X 24-EpiBr: F-ratio$_{(9 X 47)}$= 2.5327 (Significant at p d" 0.05).

Table 3 Effect of 24-EpiBr on Total sugars (mg/g fresh weight) in 60 days old plants of *B. juncea* L. plants grown under Cu stress (mean± S.E.).

Treatments	Control	0.25 mM Cu	0.5 mM Cu	0.75 mM Cu
Control	0.894 ± 0.026	1.151 ± 0.012	1.557 ± 0.068	2.001 ± 0.207
10^{-11}	0.949 ± 0.03	1.734 ± 0.05	2.457 ± 0.087	2.703 ± 0.054
10^{-9}	1.009 ± 0.061	1.952 ± 0.096	2.704 ± 0.14	2.725 ± 0.044
10^{-7}	1.098 ± 0.045	2.279 ± 0.053	2.987 ± 0.103	2.914 ± 0.022

Treatment (Cu): F-ratio$_{(3 X 47)}$= 299.825 (Significant at p d" 0.05);Dose (24-EpiBr): F-ratio$_{(3 X 47)}$= 86.887 (Significant at p d" 0.05);Cu X 24-EpiBr: F-ratio$_{(9 X 47)}$= 7.718 (Significant at p d" 0.05).

Table 4 Effect of 24-EpiBr on Reducing sugars (mg/g fresh weight) in 60 days old plants of *B. juncea* L. plants grown under Cu stress (mean± S.E.)

Treatments	Control	0.25 mM Cu	0.5 mM Cu	0.75 mM Cu
Control	1.905 ± 0.01	2.392 ± 0.011	2.504 ± 0.028	2.644 ± 0.024
10^{-11}	2.055 ± 0.014	2.429 ± 0.046	2.56 ± 0.031	2.684 ± 0.095
10^{-9}	2.083 ± 0.043	2.532 ± 0.073	2.775 ± 0.111	2.8 ± 0.095
10^{-7}	2.138 ± 0.037	2.62 ± 0.084	2.902 ± 0.095	2.819 ± 0.028

Treatment (Cu): F-ratio$_{(3 X 47)}$= 104.3 (Significant at p d" 0.05);Dose (24-EpiBr): F-ratio$_{(3 X 47)}$= 14.118 (Significant at p d" 0.05);Cu X 24-EpiBr: F-ratio$_{(9 X 47)}$= 0.805 (Non-Significant at p d" 0.05).

Table 5: Effect of 24-EpiBr on Glucose (mg/g fresh weight) in 60 days old plants of *B. juncea* L. plants grown under Cu stress (mean± S.E.)

Treatments	Control	0.25 mM Cu	0.5 mM Cu	0.75 mM Cu
Control	1.057 ±0.005	0.945 ± 0.009	0.849 ± 0.026	0.782 ± 0.014
10^{-11}	1.067 ± 0.005	0.969 ± 0.005	0.931 ± 0.005	0.83 ± 0.015
10^{-9}	1.069 ± 0.023	0.968 ± 0.006	1.016 ± 0.015	0.879 ± 0.008
10^{-7}	1.087 ± 0.012	1.06 ± 0.003	1.125 ± 0.016	0.906 ± 0.003

Treatment (Cu): F-ratio$_{(3 X 47)}$= 209.881 (Significant at p d" 0.05);Dose (24-EpiBr): F-ratio$_{(3 X 47)}$= 83.841 (Significant at p d" 0.05);Cu X 24-EpiBr: F-ratio$_{(9 X 47)}$= 13.616 (Significant at p d" 0.05).

Table 6 : Effect of 24-EpiBr on Fructose (mg/g fresh weight) in 60 days old plants of *B. juncea* L. plants grown under Cu stress (mean± S.E.)

Treatments	Control	0.25 mM Cu	0.5 mM Cu	0.75 mM Cu
Control	0.848 ± 0.007	1.446 ± 0.002	1.655 ± 0.005	1.862 ± 0.01
10^{-11}	0.989 ± 0.024	1.459 ± 0.009	1.629 ± 0.007	1.855 ± 0.006
10^{-9}	1.014± 0.008	1.564 ± 0.007	1.759 ± 0.005	1.921 ± 0.005
10^{-7}	1.052 ± 0.01	1.56 ± 0.009	1.776 ± 0.004	1.913 ± 0.011

Treatment (Cu): F-ratio$_{(3 X 47)}$= 7178.84 (Significant at p d" 0.05);Dose (24-EpiBr): F-ratio$_{(3 X 47)}$= 168.881 (Significant at p d" 0.05);Cu X 24-EpiBr: F-ratio$_{(9 X 47)}$= 15.24 (Significant at p d" 0.05).

Table 7: Effect of 24-EpiBr on Phospholipids (mg/g fresh weight) in 60 days old plants of *B. juncea* L. plants grown under Cu stress (mean± S.E.)

Treatments	Control	0.25 mM Cu	0.5 mM Cu	0.75 mM Cu
Control	0.102 ±0.001	0.089 ± 0.001	0.086 ± 0.002	0.074 ± 0.002
10^{-11}	0.1 ± 0.001	0.115 ± 0.001	0.106 ± 0	0.105 ± 0.002
10^{-9}	0.101 ± 0.001	0.12 ± 0.004	0.118 ± 0.002	0.111 ± 0.002
10^{-7}	0.11 ± 0.005	0.127 ± 0.001	0.131 ± 0.002	0.115 ± 0.001

Treatment (Cu): F-ratio$_{(3 X 47)}$= 29.933 (Significant at p d" 0.05); Dose (24-EpiBr): F-ratio$_{(3 X 47)}$= 186.785 (Significant at p d" 0.05);Cu X 24-EpiBr: F-ratio$_{(9 X 47)}$= 17.017 (Significant at p d" 0.05).

Table 8: Effect of 24-EpiBr on Glycolipids (mg/g fresh weight) in 60 days old plants of *B. juncea* L. plants grown under Cu stress (mean± S.E.)

Treatments	Control	0.25 mM Cu	0.5 mM Cu	0.75 mM Cu
Control	0.402 ± 0.004	0.503 ± 0.006	0.512 ± 0.01	0.528 ± 0.002
10^{-11}	0.409 ± 0.002	0.507 ± 0.006	0.531 ± 0	0.533 ± 0.003
10^{-9}	0.412 ± 0.003	0.508 ± 0.002	0.544 ± 0.004	0.541 ± 0.003
10^{-7}	0.428 ± 0.003	0.527 ± 0.001	0.582 ± 0.006	0.553 ± 0.011

Treatment (Cu): F-ratio$_{(3 X 47)}$= 543.591 (Significant at p d" 0.05);Dose (24-EpiBr): F-ratio$_{(3 X 47)}$= 35.099 (Significant at p d" 0.05); Cu X 24-EpiBr: F-ratio$_{(9 X 47)}$= 3.295 (Significant at p d" 0.05).

Table 9: Effect of 24-EpiBr on Total Sterols (mg/g fresh weight) in 60 days old plants of *B. juncea* L. plants grown under Cu stress (mean± S.E.)

Treatments	Control	0.25 mM Cu	0.5 mM Cu	0.75 mM Cu
Control	4.804 ± 0.043	4.309 ± 0.036	3.661 ± 0.06	2.956 ± 0.032
10^{-11}	4.709 ± 0.033	5.051 ± 0.421	4.956 ± 0.023	4.518 ± 0.031
10^{-9}	4.404 ± 0.012	4.499 ± 0.045	4.442 ± 0.052	3.698 ± 0.037
10^{-7}	4.805 ± 0.032	5.242 ± 0.054	5.68 ± 0.02	4.309 ± 0.019

Treatment (Cu): F-ratio$_{(3 X 47)}$= 496.202 (Significant at p d" 0.05);Dose (24-EpiBr): F-ratio$_{(3 X 47)}$= 675.147 (Significant at p d" 0.05);Cu X 24-EpiBr: F-ratio$_{(9 X 47)}$= 108.191 (Significant at p d" 0.05).

Table 10: Effect of 24-EpiBr on Esterified Sterols (mg/g fresh weight) in 60 days old plants of *B. juncea* L. plants grown under Cu stress (mean± S.E.)

Treatments	Control	0.25 mM Cu	0.5 mM Cu	0.75 mM Cu
Control	2.522 ± 0.022	2.134 ± 0.018	2.081 ± 0.03	2.022 ± 0.016
10^{-11}	2.594 ± 0.016	2.246 ± 0.01	2.188 ± 0.036	2.117 ± 0.015
10^{-9}	2.633 ± 0.006	2.276 ± 0.014	2.184 ± 0.018	2.122 ± 0.012
10^{-7}	2.667 ± 0.016	2.293 ± 0.027	2.289 ± 0.01	2.2 ± 0.01

Treatment (Cu): F-ratio$_{(3 X 47)}$= 422.771 (Significant at p dd 0.05);Dose (24-EpiBr): F-ratio$_{(3 X 47)}$= 45.572 (Significant at p dd 0.05);Cu X 24-EpiBr: F-ratio$_{(9 X 47)}$= 0.733 (Non-Significant at p dd 0.05).

ACKNOWLEDGEMENTS

Financial assistance from Department of Science and Technology (DST), Ministry of Science and Technology, Government of India, New Delhi, India is duly acknowledged.

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